REMARKS

Claims 15, 21-22, 25, 26 and 48-49 are pending in the application. Claims 21, 22,25 and 49 have been amended. Claim 23 has been cancelled. No new matter has been added.

Claims 21, 22 and 25 have been rejected under 37 CFR 1.74(c).

Applicants have amended claims 21, 22 and 25 and this rejection is now believed overcome.

Claims 15, 21-23, 25, 26 and 48-49 have been rejected under 35 USC 112, first paragraph.

The Examiner objects to the term "minimized" as new matter. Applicants are at a loss of why the Examiner considers the term "minimized" as new matter. "Minimized peptide" is understood by one of ordinary skill in the art that the peptide is in its lowest energy state.

This term is described at page 3, last paragraph through page 4, first paragraph and also at page 11, last paragraph. Pages 3-4, discuss that the preliminary screening of peptides for immunogenicity comprises the steps of (1) creating a molecular model of a receptor followed by minimizing the model (*meaning placing the model in the lowest energy state*) created, (2) modeling a peptide to be tested and minimizing the model of the peptide (*placing it in its lowest energy state*, then testing the fit of the model of the peptide into the model of the receptor to produce a composite minimized receptor/minimized peptide model. Upon finding an acceptable fit, the peptide may then

be screened by a binding assay for actual binding to Class II MHC as a further test for immunogenicity.

Claim 15 recites "a peptide, wherein said peptide is minimized, the minimized peptide binds to a Class II MHC receptor DR1 inhibiting the binding HA residues 306-318, the peptide selected from...(a list of peptides with specific amino acid sequences, including SEQ ID 3). Minimized, as used in claim 15, describes that the peptide is in a lowest energy state, not that it somehow pertains to peptides with sequences not listed in claim 15.

Claim 23 has been cancelled in response to the Examiner's concern that it recites 13 amino acids and the elected subject matter contains 16 amino acids.

Claims 15, 21-23, 25, 26, 48 and 49 have been rejected under 35 USC §112, first paragraph.

Claims 15, 21-23, 25, 26, 48 and 49 are directed to "an immunogenic composition." The term "immunogenic composition" was suggested by the Examiner in the personal interview conducted with applicant's representative Caroline Nash and the inventor, Dr. Reid. Applicants deleted the term vaccine and replaced it with "immunogenic composition." Support that the composition is immunogenic is found on page 13, table I and page 14, table II wherein it is shown that the CS3 pilin subunit peptide binds to the bioassay, indicating that it is immunogenic, as claimed. With the amendment of claim 21, now none of the claims recite the term "vaccine." This rejection is believed overcome.

Claims 25, 32-23, 25, 26 and 48-49 have been rejected under 35USC §112, second paragraph.

Regarding claim 15, 23 and 48, said peptide is the peptide having seq. ID no. 3. Regarding minimization, the claim only states that "wherein *when* said peptide is minimized (in its lowest energy state) it binds to a Class II MHC receptor DR1...,"

Regarding claim 21, claim 21 has been amended and does not recite vaccine.

Claims 15, 21-23 and 48 are rejected under 35 USC§102(a) as anticipated by Nauss, et al.

The present application claims priority of serial no. 08/064,559 filed May 21, 1993. A copy of the application is attached hereto with relevant portions highlighted. This priority document anti-dates the Nauss, et al article. Hence this rejection is believed overcome.

Claims 15, 21-23, 25, 26, 48 and 49 have been rejected under 35 USC §102(e) as anticipated by Reid et al. US Pat. No. 5,417,986 filed April 10, 1992. Applicant respectfully traverses this rejection.

Reid, et al. '986 discloses the use of CS3 proteins. The present application is directed to the encapsulation of CS3 peptides. The Examiner asserts that CS3 proteins necessarily contain the sequence for the CS3 peptides. This reasoning is not applicable to claim 15 and claims dependant thereon because, claim 15 requires a "**peptide, wherein when said peptide is minimized, the minimized peptide binds to a Class II MHC receptor DR1.**" The peptide is fragment of a protein and was carefully selected as a peptide that binds to the Class II MHC receptor DR1. There is no disclosure in Reid, et

al. '986 that the entire protein binds to the Class II MHC receptor DR1. To the contrary, one would not necessarily conclude that a protein would bind to the model because a peptide fragment binds to the model. The binding of the peptide to the receptor, as claimed, is space and length driven and specific. The protein is much longer than the peptide. Since Reid, et al. does not teach the specific peptide recited in claim 15 or that such a peptide binds to the Class II MHC receptor DR1 when it is minimized, it is respectfully submitted that Reid, et al. does not anticipate the claimed invention. This rejection is believed overcome.

Claims 15, 21-23, 25, 26, 48-49 have been rejected under 35 USC 103(a) as allegedly obvious over Nauss et al, Journal of Immunology, and the specification pages 12-13 in view of Reid, et al.

Nauss et al. does not anti-date the present invention for reasons stated above and thus is not a proper reference for this rejection. It is respectfully submitted that this rejection is overcome.

Versions with markings to show changes made:

Cancel claim 23.

Please amend the following claims:

21. (Twice Amended) The immunogenic composition of claim 15, wherein said composition [can be used as a vaccine component] is immunogenic against pathogenic microorganisms and neoplasms.

22. (Twice Amended) The immunogenic composition of claim [21]15, wherein said composition is immunogenic against [microorganisms is] Enterotoxogenic E. Coli.

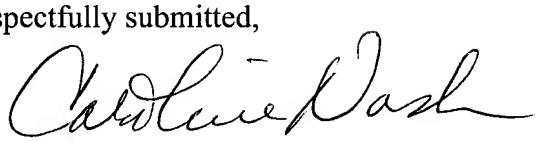
25. (Twice Amended) The immunogenic composition of claim [49]15, wherein said [immunologically] composition is combined with an immunologically acceptable carrier [acceptable carrier comprises encapsulating microspheres].

49. (Amended) The immunogenic composition of claim [15]25 wherein said [immunogenic composition is combined with an] immunologically acceptable carrier comprises encapsulating microspheres.

Reconsideration and allowance are respectfully requested.

Respectfully submitted,

Date: April 16, 2003

By 

Caroline Nash, Reg. No. 36, 329
Nash & Titus, LLC
3415 Brookeville Road, Suite 1000
Brookeville, MD 20833
(301) 924-9500

Elizabeth Arwine, Reg. No. 45,867
Attorney for Applicants
U.S. Army Medical Research
and Materiel Command
ATTN: MCMR-JA
Fort Detrick, MD 21702-5012

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APPLICANTS

JEFFREY L. NAUSS, FAIRFIELD, OH; ROBERT H. REID, KENSINGTON, MD; SCHEHERAZADE SADEGH-NASSERI, DARNESTOWN, MD.

Attachment to Amendment filed 4/16/03 in 09/013,077

CONTINUING DATA********
VERIFIED

mlr Lmlr

ABANDONED

FOREIGN/PCT APPLICATIONS********
VERIFIED

mlr Lmlr

FOREIGN FILING LICENSE GRANTED 10/13/93

Foreign priority claimed	Verified and Acknowledged	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
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35 USC 119 conditions met ☐ yes ☒ no	mlr							

ADDRESS

JOHN F. MORAN
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
COMMAND JUDGE ADVOCATE OFC., FT. DETRICK
FREDERICK, MD 21702-5012

TITLE

MODEL FOR TESTING IMMUNOGENICITY OF PEPTIDES

U.S. DEPT. of COMM./Pat. & TM Office — PTO-436L (rev. 10-78).

PARTS OF APPLICATION FILED SEPARATELY

Applications Examiner

NOTICE OF ALLOWANCE MAILED

Assistant Examiner

CLAIMS ALLOWED

Total Claims | Print Claim

ISSUE FEE

Amount Due | Dat Paid

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Sheets Drwg. | Figs. Drwg. | Print Fig.

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PREPARED FOR ISSUE

APPLICATION FOR UNITED STATES PATENT

Field of th Inv ntion:

This invention relates to a means of predicting potential of a peptide for eliciting immune response.

Background of the Invention:

Among the numerous steps required for an immunological response to occur is the presentation of the antigen by macrophages to the B-cell or T-cell. This presentation is mediated by the Class I and Class II major histocompatibility complex (MHC) molecules on the surface of the cell. The MHC molecules hold antigens in the form of the peptide fragments and together with the receptor molecule on the T-cells, form a macromolecular complex that induces a response in the T-cell. Therefore, a necessary step in an immune response is the binding of the antigen to the MHC.

Recent single crystal X-ray structures of human and murine Class I MHC's have been reported. Analysis of these crystal structures have shown that antigenic peptides lie in the so-called binding cleft for presentation to the T-cell. This cleft is formed by α_1 and α_2 domains and by ß-strands from each domain forming the floor. Furthermore, the sequence polymorphism among Class I molecules can result in alterations of the surface of the cleft forming different pockets. Peptide side chains may insert into these pockets. Thus, different pockets may interact with

different side chains. This implies the mechanism for the peptide specificity of Class I MHC's. Peptides bound to the Class I MHC's in the crystal structures were found to have both the amino and carboxy termini tightly held by the MHC. There were few interactions near the middle of the cleft. Hence the bound peptide is allowed to bend slightly in the center. The observed binding mode helped to explain the apparent partial specificity of peptide sequence and the allowed variation in peptide length found among peptides isolated from Class I MHC's.

The precise mode of binding of peptides to Class II MHC molecules is less clear. While a single crystal X-ray diffraction structure for the HLA-DR1 MHC has been shown, the coordinates have remained unavailable. However, currently available theoretical and experimental results help form a hypothesis that the binding of a peptide to Class II MHC is similar to that observed with Class I. First, it is noted that the Class II binding cleft is structurally similar to that of Class I. This was concluded based upon a sequence analysis of 26 Class I and 54 Class II amino acid sequences.

Unlike with Class I molecules, self-peptides isolated from murine I-A^b and I-E^b, from murine I-A^d and from human HLA-DR1 molecules were found to be varied in size (13 to 25 residues long). The peptides isolated from the murine I-A^b and I-E^b molecules had heterogenous carboxy termini while those from I-A^d and HLA-DR1 had ragged termini at both ends. The varying lengths indicate that the amino and carboxy termini of the peptides were

not critical for the binding. One or both termini may protrude from the binding site and be available for further processing. The r sidues critical for binding were proposed to be at the ends of the peptide as opposed to the center.

Summary of the Invention:

It is the purpose of this invention to provide a method for preliminary screening of peptides for ability to elicit antibody production. Structural homology techniques were used to model a receptor (the Class II MHC is exemplified). This model makes it possible to preliminarily screen peptides for antigenic properties. By modifying the peptide to "fit" into the receptor it is possible to identify methods of rendering non-immunogenic peptides immunogenic.

The preliminary screening of peptides for immunogenicity comprises the steps of (1) creating a molecular model of a receptor followed by minimizing the model created, 2) modeling a peptide to be tested and minimizing the model of the peptide, then testing the fit of the model of the peptide into the model of the receptor to produce a composit receptor/peptide model. It has been found that when the model of the peptide can not be fitted into the model of the receptor, the peptide will lack immunogenicity. While not all peptide models which can be made to "fit" into to model of the receptor will be effective as immunogens, the screening methods of the invention may make it possible to avoid undue biological testing of inappropriate

peptides. Bu using the model, it is also possible to alter peptides to accommodate the receptor. Hence, the invention has both predictive and drug design applications.

Brief Description of the Figures:

Fig. 1 shows the HLA-aw68 α_1 and α_2 domains with DR1 α_1 and β_1 domains.

Figs. 2-30 are a printout of the minimized coordinates of the receptor.

Detailed Description of the Invention:

In order to understand and better predict peptide interaction with Class II MHC's and as an aid for synthetic peptide vaccine design, a structural homology model of HLA-DR1 molecule was made using the Class I HLA-aw68 as a reference molecule. For purposes of this analysis, numerous conserved residues were aligned leading to a proposed three-dimensional model for the Class II structure very similar to that of Class I. This model retained the overall conformation of a Class I MHC and agreed with a considerable amount of the published data. Furthermore, peptides shown to bind to DR1 were docked in the binding cleft of the model and analyzed. The results agree with the experimental binding data presented here. Hence, it is shown that the structural homology model reported here is useful for screening Class II MHC functionality.

It had been hypothesized that few peptide residues may be required for binding to DR1. By substituting residues into the

influenza hemagglutinin 307-319 T-cell epitope it had been determined that a single tyrosine at 308 was required for binding. A synthetic peptide with the tyrosine at position 308 and a lysine at 315 was found to bind DR1 as well as the native peptide. Hence, it was concluded that few peptide residues determine the high affinity binding to DR1.

Materials and Methods:

The structural homology model for the DR1 Class II MHC was constructed using the QUANTA molecular modeling package (vision 3.2, Molecular Simulations, Inc., Burlington, MA) with the CHARMM and Protein Design modules. After alignment of the sequences as described below, gaps and loops were energy minimized using 100 steps of steepest descents minimization followed by 100 steps of adopted basis set Newton-Rapheson (ABNR) minimization. Large gaps were closed using a fragment database from a selected set of high-resolution crystal structures. The resulting structure was minimized in vacuo using 1000 steps of steepest descents followed by an additional 1000 steps of ABNR minimization. A distance related electrostatic function was used in all calculations with a dielectric constant of 1.0. Non-bound parameter lists were updated every 20 steps with a cutoff distance of 15.0Å. Non-bonded calculations were performed using a shifted potential function between 11.0Å and 14.0Å. An extended atom set was used with only polar hydrogen atoms specifically placed. There were no explicit hydrogen bond energy calculations performed.

All peptides were initially modeled using QUANTA in an

extended chain conformation and subjected to 500 steps of ABNR minimization. The resulting structures remained essentially in extended chain conformations. Individual peptides were manually docked in several different orientations into the binding cleft region of the minimized DR1 structure. The resulting bimolecular complex was subjected to 5000 steps of steepest descents minimization with non-bonded interactions updated every five steps. After minimization, bound peptides remained essentially in extended chain conformations. The lowest energy complexes for each peptide were selected for further analysis.

The selected peptide and DR1 complexes and the minimized DR1 model were subjected to the following molecular dynamics regimen: 300 steps of heating to 300K, 600 steps of equilibration at 300K, and 1100 steps of production dynamics. During this simulation, the DR1 Cα atoms were constrained in their starting positions. All non-bonded interaction parameters were as stated for the minimization procedure. The lowest energy structure during the course of the production dynamics was selected and subjected to the 5000 step minimization procedure described previously with the Cα restraints removed. The resulting structures were used for the binding energy calculations and for hydrogen bonding analysis.

Hydrogen bonds were determined using the QUANTA default parameters. Maximum allowed distances were 2.5Å between a hydrogen and the acceptor atom and 3.3Å between the donor and acceptor atoms. The minimum angle allowed between any set of

atoms forming a hydrogen bond was 90°.

Competitive Inhibition Binding Assay:

HA peptide was labeled with ^{125}I. The labeled HA peptides were then allowed to interact with purified DR1 molecules during incubation to allow formation of peptide/DR1 complexes. After incubation, the peptide/DR1 composition was exposed to a native gel for chromatographic separation of labeled peptide/DR1 complex and free labelled peptide. When unlabeled peptides were added before incubation of labeled HA peptides and DR1, and if the unlabelled peptides had capacity for binding to DR1 simultaneous with ^{125}I-HA, there was a resultant decrease in radioactive signal associated with the DR1. The extent of this decrease directly related to the binding capacity of the unlabeled unknown peptide.

Structural Homology Model for the DR1 Molecule:

The structural homology model was created, the reference molecule being the crystal structure of HLA-aw68. The HLA-aw68 coordinates and subsequent sequence were obtained from the entry 2HLA in the Brookhaven Protein Data Bank released January 15, 1991, which is incorporated herein by reference. The sequence for the DR1 molecule was for the α_1 domain was reported by Klein and for the β_1 domain, the study reported by Todd et al. (Nature 329, 599 (1987)).

The sequence alignment is based on Brown et al. (nature 332, 845 (1988)). The complete alignment and numbering scheme for both are seen in Figure 1. The Class II β_1 and Class I α_2 domains

were initially aligned on the basis of a completely conserved disulfide bridge (101 and 164 in HLA-aw68 and in DR1 cysteines 15 and 80). The remaining two domains of the DR1 protein w re not considered as they are not believed to be involved in the binding of peptides.

The α-helices were aligned in detail on the basis of conserved amino acid residues as shown in Figure 1. These conserved residues occur every three or four residues and form one face of the α-helices in both the α_1 and β_1 domains. Conversely, the polymorphic residues identified by Brown, et al line the binding cleft. The completely conserved salt bridge to stabilize the β_1 domain α-helix is also maintained by Arg 72 - Asp 76. Similarly, the ß-strand regions were also aligned, but on the basis of polymorphic regions. The conserved salt bridges His 3 - Asp 29 and Arg 111 - Asp 129 are maintained in the DR1 model with His 5 - Asp 27 in the α_1 domain and the Arg 25 - Asp 43 in the β_1 domain.

After assigning coordinates to all atoms in the DR1 model, the structure was subjected to the energy minimization procedure described previously. The resulting structure had a root-mean-square (RMS) difference in the positions of all atoms of only 0.53Å from the starting structure and in the positions of Cα atoms of only 0.33Å.

As determined through the secondary structure assignment tool in the QUANTA Protein Modeling Module, the regions of secondary structure remained essentially intact. All ß-strand

regions were conserved with som variations at the ends where the two MHC's have different loop regions. The fourth ß-strand in the α_1 domain of HLA-aw68 (residues 30-38) is disrupted in the DR1 model. Only three residues are in a ß-sheet conformation, probably due to the inserted glycine at position 28 before the strand and the large deletion in the loop region immediately after the strand. The two alpha-helical regions are clearly maintained. Both helices have been observed to be discontinuous in the Class I molecules and are similar in the DR1 model. The α_1 domain helix is long and curves from residues 49α to 76α without significant disruption. It is essentially a single continuous helix. However, the α_2 helical region is broken into two separate helices as with the Class I molecules. A short helix (52-63) is separated from a longer helix (68-94) by a deformed region without secondary structure. This deformation is more pronounced in the DR1 model as opposed to the Class I molecules due to an insertion.

Influenza Hemagglutinin Peptide with DR1:

The residues 307-319 of influenza hemagglutinin (Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr) make up a well-documented linear T-cell epitope which has been shown to be HLA-DR1 restricted. With the demonstration that the influenza hemagglutinin epitope (referred to as the HA peptide) binds DR1, it was chosen to be modeled into the binding cleft.

The peptide was initially inserted into the cleft so that Leu 11 HA was in the vicinity of the hydrophobic pocket. This

allowed Asn 7 to be near the middle charged and polar groups of the cleft. The remaining residue of the motif (Lys 2) was near the vicinity of the remaining charged and polar residues at the end of the cleft. The only adjustment to the starting conformation was a slight rearrangement of the terminal peptide proline and Tyr 3 to alleviate obvious bad contacts.

After the energy minimization of the bimolecular complex, the total energy was reduced to 483 kcal/mol (Table 1). This reduction in energy was accomplished by alleviation of several bad contacts and also be formation of several hydrogen bonds (Table 2a). The sticking feature of this mode is lack of hydrogen bonds in the carboxy terminal half of the peptide. Only one hydrogen bond is identified between the backbone carbonyl group of Leu 9 and the side chain of the β_1 Asn 77. In contrast, the amino terminal half has eleven identified interactions. Four of these interaction involve the peptide backbone residues Tyr 3, Val 4, and Gln 6. The remainder involve the side chains of Lys 2, Tyr 3, Lys 5 and Gln 6. Interestingly, Lys 5 is involved in more interactions (three) than Lys 2 (only 2). No interactions were observed as anticipated with Asn 7. Instead, it was the glutamine at position 6 donating a hydrogen bond to the α_1 Asn 62. No interactions were observed for the amino and carboxy termini.

<u>HA-YK Peptide with DR1:</u>

The binding of the HA-YK peptide (Ala-Ala-Tyr-Ala-Ala-Ala-Ala-Ala-Ala-Lys-Ala-Ala) to the DR1 model was tested. In

aligning the peptide in the cleft, it was deemed logical to insert the tyrosine residue into th hydrophobic region of the binding cleft. The lysine would then be in position to interact with the hydrophilic groups in the other half of the cleft. The resulting peptide orientation is the opposite that used for the HA and the CS3 peptides. With the peptide oriented as described, the final docking position for the peptide was unclear. The hydrophobic pocket is quite large, and, at least in this model, could accommodate the peptide tyrosine in a number of positions by sliding the peptide lengthwise through the cleft. However, repositioning the peptide also repositions the lysine. There were primarily two positions for the lysine: one with the lysine inside the cleft and the second with it outside. Of the two positions, the former was the lower in energy by 46 kcal/mol and had the greater number of interactions with the protein (11 vs. 7). Thus, the preferred orientation of the peptide appears to be with the lysine inside the binding cleft region.

CS3 subunit Pilin Peptide with DR1:

The suspected T-cell epitope for CS3 pilus subunit 63-78 (Ser-Lys-Asn-Gly-Thr-Val-Thr-Trp-Ala-His-Glu-Thr-Asn-Asn-Ser-Ala) was modeled with the DR1 molecule. The peptide was inserted with lysine 2 inside the cleft in the hydrophilic region. This placed the Thr 5 in the center of the binding cleft and the tryptophane (residue 8) near the hydrophobic region. The resulting minimized model had ten interactions between the peptide and the protein (Table 2b), three interactions with the peptide backbone and five

with the peptide side chains. The remaining two were with the amino terminal of the peptide. All of the interactions were in either the first three residues, His 10 or Glu 11 in the peptide. No interactions were observed in the center of the cleft or residues four through nine.

CFA/1 with DR1:

A peptide identified as CFA/1 (Val-Gly-Lys-Asn-Ile-Thr-Val-Thr-Ala-Ser-Val-Asp-Pro) was prepared and an attempt was made to "fit" the molecule into the cleft of the DR1. The lysine at position 3 prevented insertion of the peptide.

Results:

The peptides chosen to dock in the DR1 model are shown in Table 1. The peptides were docked manually in several orientations into the DR1 model. The peptides were then tested in biological binding assays with the following results:

Table I

Peptide	Molecular model predicted binding	Binding in the bioassay
HA (influenza hemagglutinin)	Yes	Yes
HA-YK (synthetic peptide)	Yes	Yes
CS3 pilin subunit	Yes	Yes
CFA/1	No	No

CLAIMS

1. A method of preliminarily screening peptides for immunogenicity comprising the steps of:

   1) creating a molecular model of receptor DR1 Class II MHC and minimizing the model of the DR1;

   2) modeling a peptide to be tested and minimizing the model of the peptide; and

   3) testing fit of model obtained in step 2 into the model obtained in step 1 to produce a composite receptor/peptide model.

2. A computerized model comprising a model of the DR1 molecule having fitted in a cleft therein a model of a peptide.

3. A method of claim 1 wherein, additionally, the receptor/peptide model is subjected to computer-simulated heating.